IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Cindie M. Luhman	
Serial No.	:	09/239,873	
Filed	:	January 29, 1999	Group Art Unit: 1616
For	:	METHOD AND COMPOSITION FOR ENHANCING MILK COMPONENT CONCENTRATIONS	Examiner: N. Levy
Docket No.	:	LL11.12-0040	

#19
attarmon
6/20/02

DECLARATION UNDER 35 U.S.C. §132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

SENT VIA EXPRESS MAIL
Express Mail No.: EV030213287US

Sir:

I, Paul A. Porter, of 1025 190th Street, Webster City, Iowa, hereby declare as follows:

1. I am currently the Dairy Research Manager of the Land O' Lakes Research Farm in Webster City, Iowa.

2. I obtained a Bachelor of Science (B.S.) degree in Chemistry from Wittenberg University of Springfield, Ohio in 1981.

3. I obtained a Masters of Science (M.S.) in Animal Science from Cornell University in Ithaca, New York in 1984.

4. I obtained a Doctorate of Philosophy (Ph.D.) in Animal Science, with an emphasis in Dairy Nutrition, from Cornell University in Ithaca, New York in 1987.

5. Since completing my Doctorate of Philosophy degree in 1987, I have continuously been employed in positions directly relating to dairy herd management and nutrition.

6. From 1987 to 1989, I was an Assistant Professor in the Department of Animal Science at Oregon State University in Corvallis, Oregon, where I conducted applied dairy nutrition research; taught courses in ration balancing. dairy herd management, and advanced dairy herd management; and served as a liaison to the Oregon State Dairy Industry.

7. From 1989 to 1992, I was the Dairy Program Manager for Countrymark Co-op of Delaware, Ohio, where I provided technical assistance, troubleshooting expertise, product support, and training to local dairy cooperatives, sales staff of the local dairy cooperatives, and dairy farm clients of the local dairy cooperatives.

8. From 1992 to 1994, I provided dairy sales and dairy consulting for Young's Livestock Nutritional Services of Canastota, New York, where I analyzed dairy production records, consulted on dairy production issues, and designed customized dairy herd feeding programs for dairy farm clients.

9. From 1994 to 2000, I was Dairy Nutritionist and Field Technical Services Manager for Land O' Lakes Dairy Feed of Sun Prairie, Wisconsin, where I provided technical assistance, Troubleshooting services, product support, and training to local dairy cooperatives, sales staff of the local dairy cooperatives, and dairy farm clients of the local dairy cooperatives.

10. From 2000 to the present, I have been Dairy Research Manager for Land O' Lakes Research Farm of Webster City, Iowa, where I am responsible for design, implementation, and summarizing dairy research trials for the farm's dairy herd that includes 240 cows. Additionally, as Dairy Research Manager, I assist in new feed product development and coordinate with the Feed Division of Land O' Lakes, Inc. regarding feeding trial results. Finally, as Dairy Research Manager, I am responsible for my group's annual budget of approximately $700,000.00.

11. A brief resume of my educational career and my professional career from 1981 to the present is attached hereto as Exhibit A.

12. I am experienced in dairy herd feeding experiments and trials, including both *in vitro* and *in vitro* approaches, due my education and work experience relating to dairy nutrition issues over the past 18 years.

13. The term *in vitro* describes "a biological reaction which can be performed outside the living organism in the laboratory; as, in a test tube or petri dish, on a microscope slide, etc.", whereas the term *in vivo d*escribes "a reaction which takes place within the living organism," according to Grant, Roger and Grant, Claire, Grant & Hackh's Chemical Dictionary, page 307 (5th edition McGraw-Hill Book Company), which is attached to this Declaration as Exhibit B.

14. *In vivo* experiments exploring the function of microorganisms in the rumen of living animals and *in vitro* experiments exploring the function of rumen microorganisms in a laboratory and isolated from the rumen of the animal are described in Hobson,

P.N. The Rumen Microbial Ecosystem, pages 461-463 (1998, 1st Edition, Elsevier Science Publishers Ltd.), which is attached to this Declaration as Exhibit C. Exhibit C further indicates that *in vitro* results are not always reproducible under *in vivo* conditions in the rumen of a ruminant.

15. Similarly, Hobson, P.N. and Stewart C.S., The Rumen Microbial Ecosystem, pages 661-662 (1997, 2nd Edition, Chapman and Hall), which is attached to this Declaration as Exhibit D, states that *in vitro* conditions "might reproduce metabolic pathways of rumen microorganisms" that occur under *in vivo* conditions, but do not always or necessarily reproduce *in vivo* results.

16. As support for the facts provided in Paragraph 15 of this Declaration, Exhibit D states that *in vitro* experiments "could not always" quantitatively reproduce metabolic reactions of rumen microorganisms observed under *in vivo* conditions and further states: "The only 'container' that reproduces the rumen is the rumen."

17. Exhibit C posits some reasons why rumen microorganism reactions under *in vitro* conditions do not always reproduce rumen microorganism reaction results observed under *in vivo* conditions. For example, Exhibit C states:

> The fact that the reactions *in vitro* do not reproduce those *in vivo* does not necessarily mean that the test organism has no place in the rumen system; it may be that it's growth conditions in the rumen have not been properly reproduced in the laboratory, or it may mean that it's growth in the rumen is overshadowed by some unisolated organism."

18. Indeed, as observed in Hobson, P.N., and Stewart, C.S., The Rumen Microbial Ecosystem, page 496 (1997, 2nd Edition, Chapman and Hall), which is attached to this

Declaration as Exhibit E, the rumen is a complex system with many interrelated sub-systems:

> The rumen has a complex structure and the contents are heterogeneous, consisting primarily of a microbial suspension in free liquid, a solid mass of digesta, and a gas phase. Each of these entities is complex; the properties of microorganisms in suspension deep in the rumen might be different from those of microorganisms close to the rumen wall (Cheng and Costerton, 1980.) The solid mass of digesta is certainly heterogeneous, and even the gas phase is complex, consisting of a large gas space (gas-cap), smaller pockets of gas entrapped within the solid mass, and the dissolved gas. Many properties of individual parts of the microbial system of the rumen have been investigated, but often the investigations were governed by the ease of experimentation rather than the importance of the given component. This is why the complex, and relatively inaccessible, semi-solid mass of digesta has received so little attention. In addition, worthwhile investigations were hampered by a lack of simple conceptual basis for dealing with such a complex system.

19. Further factual explanation about the inconsistencies and variations observed between *in vitro* results versus *in vivo* results in relation to rumen metabolism, as mentioned in Paragraphs 14 to 17 above, is provided in Hobson, P.N. and Stewart, C.S., The Rumen Microbial Ecosystem, page 518 (1997, 2nd Edition, Chapman and Hall), attached to this Declaration as Exhibit F, which states:

> The microbial system of the rumen is not a simple fermentation vat filled with randomly distributed mixtures of microorganisms. It is a highly structured and functionally compartmented system, on a par with any of the organs of the advanced multicellular organism. Significant progress in the field of rumen metabolism is contingent on appreciation of the rumen and its microbial ecosystem in this manner.

20. Continuing, Hobson, P.N., The Rumen Microbial Ecosystem, page 428 (1988, 1st Edition, Elsevier Science Publisher's Ltd.), which is attached to this Declaration as Exhibit G, explains that complex "interrelations within the system of rumen metabolism," such as "microbial growth yield" issues and "digesta kinetics" issues make "the study of rumen optimization a very laborious task" and suggests that this complexity of interrelations within the rumen metabolism system is the primary reason for variability and contradictory results between *in vitro* and *in vivo* systems regarding rumen metabolism:

> The complexity of the rumen and the ruminant systems to be manipulated is the main reason for variability and contradiction in experimental results.

21. As one example of the variability that may be expected between *in vitro* and *in vivo* experimental results relating to rumen metabolism, Hobson, P.N. and Stewart C.S., The Rumen Microbial Ecosystem, pages 586-587 (1997, 2nd Edition, Chapman and Hall), which is attached to this Declaration as Exhibit H, states that fiber digestibility upon isoacid supplementation showed a positive response during under *in vitro* conditions, but showed only a minimal or insignificant response under *in vivo* conditions during a rumen metabolism study.

22. Next, Hobson, P.N., The Rumen Microbial Ecosystem, pages 140-141 (1988, 1st Edition, Elsevier Science Publishers Ltd.), which is attached to this Declaration as Exhibit I, observed that added haem created a zoosporogenesis under *in vivo* rumen conditions, "but this could not be satisfactorily repeated *in vitro* with pure cultures, suggesting that the control of zoosporogenesis and zoospore really may be more complex than suggested by the work *in vivo*." (Emphasis added.)

23. Finally, even where rumen system complexity is not a factor, Santoro, Luiz G., Grant, George, and Pusztai, A. Arpad, Differences in the Degradation *in Vivo* and *in Vitro* of Phaseolin, the Major Storage Protein of *Phaseolus Vulgaris* Seeds, pages 612-613, Biological Society Transactions - 625th Meeting (London 1998), which is attached to this Declaration as Exhibit J, demonstrates that large quantitative variations between *in vivo* and *in vitro* nevertheless can occur. Specifically, in the study that was the subject of Exhibit H, digestive degradation of phaseolin (glycoprotein II, G-II) under *in vivo* conditions was about 74%, while the extent of digestive degradation of phaseolin under *in vitro* conditions in hamsters was only about 2%, which is vastly different from the results obtained under *in vivo* conditions.

24. Thus, the collective teaching of the factual observations and statements in Paragraphs 14 to 22 above is that *in vitro* simulations of rumen metabolism, at least from a quantitative perspective, and possibly from other perspectives, are not a reliable predictor of *in vivo* rumen metabolism results, but are instead speculative. Furthermore, the factual observations and statements of Paragraphs 14 to 22 above demonstrates that, for the same substrate, widely different quantitative results may be observed when comparing *in vitro* rumen metabolism experimental results to actual *in vivo* rumen metabolism results.

25. I am familiar with the disclosure of U.S. Patent No. 4,127,676 to Merensalmi (the "Merensalmi Patent"), which is attached to this Declaration as Exhibit K.

26. In Example 1, the Merensalmi Patent presents *in vitro* testing results regarding sugar alcohol preservation "in rumen fluid." (Column 2, lines 59-63, of the Merensalmi Patent of Exhibit K).

27. The Merensalmi Patent does not provide any details whatsoever regarding the source, composition, handling, or preservation of the rumen fluid used in the *in vitro* testing referenced in paragraph 26 above and does not provide any details whatsoever about the experimental protocol or procedure for the Example 1 *in vitro* testing of sugar alcohol preservation in "rumen fluid."

28. Nonetheless, despite providing no evidence about the *in vitro* testing protocol or procedure and despite providing no evidence about the source, composition, handling, or preservation of the rumen fluid, the Merensalmi Patent nevertheless alleges that the *in vitro* testing results of Example 1 conclusively establish that similar sugar alcohol degradation rates to those presented in Example 1 *would* occur under *in vivo* conditions in the rumen of a living ruminant:

> The present invention, however, is based upon the prior unknown fact that sugar alcohols remain intact also under the conditions in the rumen sufficiently long without breaking down, <u>which appears from the</u> [*in vitro*] <u>test results presented in Example 1</u>."

(Column 2, lines 53-57, of the Merensalmi Patent of Exhibit K; emphasis added).

29. Furthermore, the Merensalmi patent provides no evidence or reasoning whatsoever in support of the statement recited in Paragraph 28 above alleging that the *in vitro* testing results of Example 1 conclusively establish that similar sugar alcohol

degradation rates to those presented in Example 1 *would* occur under *in vivo* conditions in the rumen of a living ruminant.

30. Based upon the collective teaching of the factual observations and statements in Paragraphs 14 to 22 above about *in vitro* simulations of rumen metabolism, at least from a quantitative perspective, <u>not</u> being a reliable predictor of *in vivo* rumen metabolism results, and based upon my factual observations in Paragraph 28 and 29 above, the allegation recited from the Merensalmi patent in Paragraph 28 above is simply an unsupported and inconclusive allegation that does <u>not</u> actually disclose, or support a conclusion, that similar sugar alcohol degradation rates to those presented in Example 1 *would* occur under *in vivo* conditions in the rumen of a living ruminant. Instead, the factual observations and statements of Paragraphs 14 to 22 above demonstrate that, for the same substrate, such as the individual sugar alcohols of Merensalmi Example 1, widely different quantitative results may be observed when comparing *in vitro* rumen metabolism experimental results to actual *in vivo* rumen metabolism results.

31. Building upon my factual observations of Paragraph 30, the Merensalmi Patent approach of equating the *in vitro* sugar alcohol degradation results of Merensalmi Example 1 to real life *in vivo* sugar alcohol degradation results that allegedly *would* be obtained under *in vivo* conditions using the complex rumen function of a live ruminant is mere speculation, since "The only 'container' that reproduces the rumen is the rumen." (See Exhibit D).

32. Indeed, based upon my factual observations and factual conclusions referenced in Paragraphs 27 and 29 to 31 above, the Merensalmi tactic of equating the *in vitro* results from Merensalmi Example 1 to results that *would allegedly* be obtained under *in vivo* conditions is speculative at best and does not in fact disclose, establish, or prove sugar alcohol degradation rates that would be experienced under *in vivo* conditions.

33. Based on my factual observations of paragraph 32 above, it follows that the tabular *in vitro* results that extend from column 2, line 65, through column 3, line 5, of the Merensalmi Patent pertain purely to the results of the Merensalmi *in vitro* testing under unspecified conditions and an unspecified testing protocol and do not establish, prove, or disclose anything about sugar alcohol degradation in the rumen of a living ruminant under *in vivo* conditions.

34. Example 2 , which extends from line 12 to line 38 in column 3 of the Merensalmi Patent, provides additional test results for a sugar alcohol mixture, as opposed to the test results provided in Example 1 of the Merensalmi Patent for individual sugar alcohols.

35. Example 2, like Example 1, refers to testing "in the rumen fluid," as opposed to *in vivo* testing in the rumen of a live ruminant, and therefore, like the test results of Example 1, merely amounts to *in vitro* experimentation about the sugar alcohol mixture degradation characteristics in "the rumen fluid."

36. The Merensalmi Patent does not provide any details whatsoever regarding the source, composition, handling, or preservation of the rumen fluid used in the *in vitro* testing referenced in paragraphs 34 and 35 above and does not provide any details whatsoever about the experimental protocol or procedure for the Example 2 *in vitro* testing of the sugar alcohol mixture preservation in "rumen fluid."

37. Furthermore, the Merensalmi patent provides no evidence or reasoning whatsoever that would support an allegation that the *in vitro* testing results of Example 2 conclusively establish that similar sugar alcohol degradation rates to those presented in Example 2 *would* occur under *in vivo* conditions in the rumen of a living ruminant.

38. Despite the complete lack of protocol, procedure, reasoning and evidence, Merensalmi Example 2 as with Example 1, alleges that the results of the Example 2 *in vitro* testing equate to results that *would* be obtained if the sugar alcohol mixture were instead subjected to *in vivo* testing in the rumen of a living ruminant:

> As the flow of the fluid in the rumen is only a few hours, the sugar alcohol reaches the latter stomach compartments before any essential degradation in the rumen can occur.

(Column 3, lines 30-33, of the Merensalmi Patent of Exhibit K).

39. Nonetheless, despite this Merensalmi allegation that is recited in Paragraph 38 above, the results of the *in vitro* testing of Example 2, for reasons analogous to those provided in Paragraphs 30 to 32 above with regard to Merensalmi Example 1, do <u>not</u> establish, prove, or disclose sugar alcohol degradation rates under *in vivo* conditions in the rumen of a living ruminant and do not establish, prove, or disclose sugar alcohol degradation rates that *would* necessarily be expected upon replacing the *in*

vitro procedure of Example 2 with a true *in vivo* procedure in the rumen of a living ruminant.

40. Instead, for reasons analogous to those provided in Paragraphs 30 to 32 above with regard to Merensalmi Example 1, the attempt of Merensalmi Example 2 to equate the *in vitro* sugar alcohol degradation rate results to *in vivo* results is purely speculative and without any evidence or basis in fact.

41. Finally, the last sentence in Example 4 of the Merensalmi Patent states:

> The major part of the sugar alcohol mixture however passes through the rumen without breaking down.

(Column 4, lines 45-47, of the Merensalmi Patent of Exhibit K).

42. However, the only basis for this statement of Merensalmi Example 4 that is recited in Paragraph 38 above, are the erroneous and unsupported allegations of the Merensalmi patent regarding Examples 1 and 2 that are mentioned in Paragraphs 28 and 38 above

43. Consequently, for reasons analogous to those provided in Paragraphs 30 to 33 and 39 to 40 above, there is no evidence or factual basis in support of the Merensalmi Example 4 statement that is recited in Paragraph 43 above; consequently, for reasons analogous to those provided in Paragraphs 30 to 33 and 39 to 40 above, the statement recited in Paragraph 43 above regarding Merensalmi Example 4, like the prior Merensalmi statements equating the *in vitro* results of Examples 1 and 2 to *in vivo* results, is purely speculative and without any basis in fact.

44. Ultimately, considering the factual statements and factual observations provided in Paragraphs 26 to 43, the Merensalmi patent only provides details about *in vitro* sugar alcohol degradation testing under unspecified conditions and an unspecified testing protocol and doe not establish, prove, or disclose anything about sugar alcohol degradation in the rumen of a living ruminant under *in vivo* conditions.

I declare that all statements made herein that are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Inventor: Paul A. Porter
(Printed Name)

Inventor: Paul A Porter
(Signature)

Date 6/3/02

Applicant	:	Cindie M. Luhman	
Serial No.	:	09/239,873	
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For	:	METHOD AND COMPOSITION FOR ENHANCING MILK COMPONENT CONCENTRATIONS	Examiner: N. Levy
Docket No.	:	LL11.12-0040	

EXHIBIT A

of

DECLARATION UNDER 35 U.S.C. § 132

Resume of the Declarant, Paul A. Porter

Paul A. Porter, Ph.D.
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Webster City, IA 50595
515 543-4852 x222

Education:

Ph.D., Animal Science (Dairy Nutrition emphasis), Cornell University, Ithaca, NY. 1987.
M.S., Animal Science, Cornell University, Ithaca, NY. 1984.
B.A., Chemistry, Wittenberg University, Springfield, OH. 1981.

Professional Experience:

2000-present
Dairy Research Manager, Land O' Lakes Research Farm, Webster City, IA
Responsible for design, implementation and summary of research trials in the 240-cow dairy herd; present summarized information to the Feed Division and assist in new product development; manage $700,000 annual budget.

1994-2000
Dairy Nutritionist and Field Technical Services Manager, Land O' Lakes Dairy Feed, Sun Prairie, WI
Provided technical assistance, troubleshooting, product support and training to local cooperatives, their sales staff and their dairy farm clients. Developed sales tools such as spreadsheet economic evaluations and new product information materials.

1992-1994
Dairy Sales & Consulting, Young's Livestock Nutritional Services, Canastota, NY
Provided management and production record analysis & consulting and designed customized feeding programs for dairy farm clients packaged with sales of mineral products.

1989-1992
Dairy Program Manager, Countrymark Coop, Delaware, OH
Provided technical assistance, troubleshooting, product support and training to local cooperatives, their sales staff and their dairy farm clients. Developed sales tools such as spreadsheet economic evaluations and new product information materials.

1987-1989
Assistant Professor, Department of Animal Science, Oregon State University, Corvallis
Conducted applied dairy nutrition research and taught courses in ration balancing, dairy herd management and advanced dairy herd management; acted as liaison to state dairy industry and presented papers at numerous meetings.

Memberships:
American Dairy Science Association
American Registry of Professional Animal Scientists (ARPAS)

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EXHIBIT B

of

DECLARATION UNDER 35 U.S.C. § 132

Grant, Roger and Grant, Claire, Grant & Hackh's Chemical Dictionary, page 307 (5th edition McGraw-Hill Book Company)

intramuscular Inside muscular tissue; as, of an injection.
intranuclear (1) Within an atomic nucleus. (2) Within a molecular ring system. **i. tautomerism** The shifting of a double bond within one or more rings.
Intraval sodium Trademark for thiopental sodium.
intravenous Within veins, e.g., an injection.
intravital (1) Within the living organism. (2) Within a lifetime.
introduction Causing the entry of a different type of atom into an organic molecule, e.g., chlorination.
introfaction A change in the fluidity and specific wetting properties of an impregnating material, due to an introfier.
introfier Impregnation accelerator. A substance that speeds up the penetrating power of fluids.
intrusion Forcing a material into the cavities or pores of a substance.
intumescence (1) Swelling up, especially of certain crystals on heating. (2) Popping, puffing. The violent escape of moisture on heating.
inula camphor Helenin.
inulenin $(C_6H_{10}O_5)_n \cdot 2H_2O$. A carbohydrate associated with inulin. Colorless needles, soluble in water.
inulic acid Alantic acid.
inulin $C_6H_{11}O_5(C_6H_{10}O_5)_nOH$. Alantin, alant starch, dahlin, sinistrin. A polysaccharide from the rhizome of *Inula helenium* or *Dahlia variabilis*. White powder, m.160 (decomp.), soluble in hot water. Used to measure glomerular filtration rate of kidneys.
inulinase* An enzyme that endohydrolyzes 2,1-β-D-fructosidic linkages in inulin.
in vacuo In a *vacuum*, q.v.
Invar Trademark for the ferronickel: Ni 36, steel 64% (carbon content 0.2%), d.8.0, m.1500. It has a low coefficient of heat expansion; used for precision instruments.
inversion (1) The turning of a levo to a dextro compound, or vice versa. (2) The change of an isomeric compound to its opposite, as a *cis* to a *trans* compound. (3) The hydrolysis of an optically active disaccharide to 2 optically active monosaccharides; e.g., the hydrolysis of cane sugar to glucose and fructose by dilute acids, alkalies, or enzymes, resulting in a change in the direction and degree of rotation of polarized light. Cf. *Walden inversion, Clerget inversion.* (4) In an emulsion of 2 immiscible liquids, the interchange of the internal and external phases. **dipole ~** Symmetrization. The reversal of the normal activity of functional groups in organic chemistry.
i. point The temperature at which i. takes place.
invertase β-D-Fructofuranosidase*, saccharase, invertin. An enzyme of the pancreatic juice and of yeast, which hydrolyzes terminal, nonreducing β-D-fructofuranoside residues in β-D-fructofuranosides; converts cane sugar into invert sugar.
invertin Invertase.
invert soap A cationic, surface-active detergent, so called because it ionizes oppositely to soap; e.g., quaternary ammonium or sulfonium compounds.
invert sugar Approximately 50% glucose and 50% fructose, obtained by the acid hydrolysis of cane sugar. It is slightly levorotatory, fermentable; it reduces Fehling's solution and is used in brewing. **i. s. solution** A partially inverted solution of sucrose containing at least 62% solids, 3–50% i. s., and equal weights of fructose and glucose.
in vitro Describing a biological reaction which can be performed outside the living organism in the laboratory; as, in a test tube or petri dish, on a microscope slide, etc. Cf. *in vivo.* **i. v. fertilization** I.V.F. Fertilization in the laboratory of a (human) ovum, removed from an ovary, by sperm. (Used in conception of "test tube babies.") See *embryo replacement.*
in vivo Describing a reaction which takes place within the living organism. Cf. *in vitro.*
inyoite $2CaO \cdot 3B_2O_3 \cdot 13H_2O$. A native borate (S. California).
iod- See *iodo-*.
iodal $CI_3 \cdot CHO$ = 421.7. A liquid resembling chloral.
iodalbin A red compound of blood albumin and iodine, of molasseslike odor.
iodaniline Iodoaniline*.
iodate* A salt of iodic acid, containing the radical IO_3^-.
iodeosin $C_{20}H_8O_5I_4$ = 835.9. Erythrosin, tetraiodofluorescein. A red indicator powder, soluble in alcohol (alkalies—rose-red, acids—yellow). **i. solution** A 0.0002% solution of iodeosin in ether. This is added to dilute alkali and titrated until the rose tint passes from the ether into the aqueous solution.
iodi- See *iodo-*.
iodic **i. acid*** HIO_3 = 175.9. Metaiodic acid. Colorless rhombs, m.110, soluble in water. Used as an oxidizing agent; as a reagent for alkaloids, biliary pigments, naphthol, thiocyanates, and guaiacol; in organic synthesis, and for volumetric solutions **per ~** See *periodic acid.* **i. anhydride** Iodine pentaoxide*.
iodide* MI_n. A binary compound of iodine with a metal. **i. ion*** The I^- ion.
iodimetry Iodometry.
iodinated (^{131}I) serum A sterile solution of human serum albumin, treated with ^{131}I and freed from iodide; used to diagnose lung conditions; as, small tumors or emboli, and to estimate blood volume.
iodine* I = 126.9045. Id* (if I* is inconvenient). Iodum. A nonmetallic element, at. no. 53, of the halogen group. Rhombic, bluish-black, lustrous plates or scales, d.4.948, m.114, b.184, slightly soluble in water, soluble in alcohol or iodide solutions. Discovered by Courtois (1811) and named after its purple vapors (Greek: *iodes*, the "violet" and *ion*, "similar"). Obtained from the mother liquor of Chile saltpeter and seaweed ash, and widespread in nature. Valency: usually 1 (iodides*), or 3 (iodonium*), or 5 (iodates*). Used as a reagent in volumetric analysis; in organic synthesis; in the manufacture of iodides, iodates, and iodine preparations; and as an antiseptic and caustic. Used medically (^{125}I and ^{131}I) as sodium iodide and iodinated albumin. I. is also an essential trace element, present in thyroid hormones; deficiency in diet leads to goiter and hypothyroidism. Recommended daily intake 150 μg. **eka ~** Early name for astatine. **povidone- ~** $(C_6H_9ON)_nI$. 1-Vinyl-2-pyrrolidinone polymer with iodine. Betadine. An antiseptic (USP, EP, BP). **solution of ~** (1) Lugol solution. (2) Colorless Lugol solution; decolorized with sodium thiosulfate. (3) *Iodine water*, q.v. **tincture of ~** An alcoholic 7% iodine solution in 5% potassium iodide solution; an antiseptic (USP).
i. acetate* $IC_2H_3O_2$ = 185.9. A solid prepared from chlorine dioxide and i. in glacial acetic acid. **i. bromides** IBr, i. monobromide; IBr_3, i. tribromide; IBr_5, i. pentabromide. **i. chlorides** ICl, i. monochloride; ICl_3, i. trichloride. **i. cyanide*** ICN = 152.9. Cyanogen i. Colorless crystals, m.146, soluble in water. **i. cycle** See Fig. 15. **i. dioxide*** IO_2 = 158.9 or I_2O_4 = 317.8. Yellow powder, decomp. into its elements at 130. **i. disulfide** Sulfur iodide. **i. green** A phenolphthalein dye pH indicator, changing at 1.0 from yellow (acid) to blue-green (alkaline); also stains liquefied xylem in plant tissues. **i. fluoride** See *iodine pentafluoride.* **i. monobromide*** IBr = 206.8. Purple crystals, m.36, soluble in water (decomp.). Used

GRANT & HACKH'S

CHEMICAL DICTIONARY

[American, International, European and British Usage]

Containing the Words Generally Used in Chemistry, and Many of the Terms Used in the Related Sciences of Physics, Medicine, Engineering, Biology, Pharmacy, Astrophysics, Agriculture, Mineralogy, etc.

Based on Recent Scientific Literature

FIFTH EDITION
Completely Revised and Edited by

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The previous edition of this book was *Hackh's Chemical Dictionary*, 4th ed., published by McGraw-Hill in 1969. It was prepared by Dr. Julius Grant from a *Chemical Dictionary* compiled by Ingo W. D. Hackh. The current, or 5th, edition of this book was prepared by Dr. Roger L. Grant, whose father prepared the 4th edition.

The editors for this book were Betty J. Sun and Susan Thomas, the designer was Naomi Auerbach, and the production supervisor was Teresa F. Leaden. It was set in Palatino by University Graphics, Inc.

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EXHIBIT C

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DECLARATION UNDER 35 U.S.C. § 132

Hobson, P.N., The Rumen Microbial Ecosystem,
pages 461-463 (1988, 1st Edition, Elsevier Science Publishers Ltd.)

15

Models, Mathematical and Biological, of the Rumen Function

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Honorary Research Fellow, Biochemistry Department, Marischal College, Aberdeen University, Aberdeen, UK

Formerly Head, Microbial Chemistry Department, Rowett Research Institute, Aberdeen, UK

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J.-P. Jouany

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TYPES OF MODELS

The biological model

In the preceding chapters the functions of the rumen microorganisms as a whole and in the animal, of the microorganisms transferred to a laboratory vessel, and of microorganisms isolated from the rumen mixture acting alone or with one or two others in laboratory apparatus, have been described. It is initially assumed in the experiments *in vitro* that the microbes will react as they do in the rumen. Some comparison of the results with measurements made on the organisms *in vivo* is then made to see how far the initial assumption can be verified. The fact that the reactions *in vitro* do not reproduce those *in vivo* does not necessarily mean that the test organism has no place in the rumen system; it may be that its growth conditions in the rumen have not been properly reproduced in the laboratory, or it may mean that its growth in the rumen is overshadowed by some unisolated organism. On the other hand, the fact that the rumen reactions are apparently reproduced *in vitro* does not always mean that the test organism has a place in the rumen flora and that it is there behaving as in the laboratory experiment. Pure cultures of many organisms may react similarly, as may mixed cultures containing a variety of components.

These cultures may be referred to as 'biological models' of the rumen system. However, they are, probably invariably, reproducing only a part of the rumen reactions. This may be by design; a pure culture or mixed culture of two or three components cannot be expected to reproduce more than the reactions it was

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designed to study. On the other hand, it may not be safe to assume that a complex mixed culture, such as an artificial rumen, is reproducing all features of the natural rumen because it is reproducing the main features.

One of the assumptions inherent in the tests with pure, or simple defined, cultures is that, if the reactions *in vitro* and *in vivo* are very similar, the test organisms must be those which are carrying out the rumen reactions, or else that any other organisms concerned are very similar to the test organisms, and that the rumen reaction requires these organisms alone. As a corollary, it is assumed that if the complex mixed culture, the artificial rumen, functions in its measured parameters as does the rumen although it is known that some organisms present in the natural rumen are not found in the artificial habitat, then these organisms are not necessary to the proper function of the natural system.

Various experiments, of the type just discussed and others, suggested that the functional rumen population was composed of a limited number of organisms and that the others in the complex population that exists in the actual rumen were unnecessary or there only by chance, possibly kept growing by continued inoculation from the animal's surroundings. Undoubtedly there are some organisms, common to soils and waters for instance, that are always present in the rumen in low numbers but to which, because of their properties and the numbers in which they occur, no reasonable function can be assigned (see Chapter 2). Indeed, their properties may make it reasonable to assume that their apparent growth is only the result of continued inoculation. This assumption is probably quite true for organisms present in only tens or hundreds per ml of rumen contents. It is more difficult to prove this hypothesis for organisms which are present in numbers of some millions per ml, even though this number is only a fraction of 1% of the rumen bacterial population of some 10^{10} per ml.

Whether or not the biological model is reproducing *in vitro* the reactions of the rumen flora, it cannot physically reproduce the rumen and its functions in association with the animal. Because of contamination from its surroundings, the normal animal cannot be used to study the hypothesis of the limited functional rumen population. Thus the idea of using ruminants brought up in a sterile environment, with no rumen microbes, to which could be given a defined rumen flora, came about. This is the 'gnotobiotic lamb' concept. The functions of a rumen population *in toto* could be investigated not only by measuring certain parameters but by assessing others in the ability of the flora to keep the animal alive and healthy. The gnotobiotic lamb is still a 'model' in that it seeks to reproduce the rumen function without reproducing the natural rumen flora. It can also be used to model such things as the natural invasion of the rumen by animal-pathogenic organisms, or the involvement of certain bacteria in rumen malfunction or detoxification of certain toxic feeds (Chapter 14). However, unlike the *in vitro* model, the *in vivo* model must reproduce the whole digestive function to keep the animal alive, even if the main objective is to study only part of the system.

To test the functions of the rumen ciliate protozoa, a rather less rigorously controlled biological model than the gnotobiotic lamb can be used, and the

production of, and the results obtained with, the two types of model will be described later in this chapter.

The mathematical model

The rumen system is an 'open' microbial culture. A mass of microbial nutrients, in the animal feed, enters the rumen at intervals. In the rumen the nutrients are degraded to various extents to different end-products which may be absorbed from the rumen or pass on with undegraded feed components to the more distal portions of the gut. The microorganisms grow, die, lyse or pass on with the undegraded feed. Liquid, as saliva or drinking water, also enters and leaves the rumen. The system is thus concerned with changes in weights, of microbes and feedstuffs and products, and with rates of degradation and synthesis, and passage into and out of the system. All these changes are related, and it should theoretically be possible to make expressions describing the relationships and use these expressions to determine the effects of changes in one factor on another. This is a 'mathematical' model of quantitative functions of the rumen microbes. The first step in making a mathematical model is to be able to describe the system in words or a diagram. This then should suggest the data which will be needed to evaluate quantitatively the changes of the description. The model may not describe the whole system; this may be impossible or not necessary, but if the whole is not described then it should be remembered that the rest of the system may affect the part described.

Over many years various mathematical expressions have been developed which try to describe the growth and metabolism of bacteria under different conditions. These expressions vary in complexity and the extent to which they describe the bacterial functions (see, for instance, Bazin, 1983; Roels, 1983). This is partly because of theoretical limitations and partly because of the uses to which the expressions have been put. A simple description of oxygen uptake rates which does not concern itself with the mechanisms of oxygen metabolism may be quite sufficient in fermentor control, for instance, and one of the reasons for mathematical modelling is to produce control systems for microbial fermentations. Another of the reasons for mathematical modelling of bacterial growth and metabolism is that the success of the model in describing the actual behaviour of bacteria in culture can help to determine the extent of our knowledge of the processes which go on in the bacterial cell and in populations of bacteria. A further, and more usual, reason is that the model hopes to describe the system with such accuracy that it can replace experiments with the actual system and predict what will happen if some controlling parameters of the system are changed. Thus a rumen model might hope to predict what would happen to production of VFA or other metabolites if feed was changed in quantity or quality. However, the complexity of a rumen model will depend on the use to which the model is to be put as well as the state of knowledge of the microbial system. The mathematical model is like the *in vitro* fermentations with pure or mixed rumen bacteria in that it can describe the whole or only a part of the digestive process, and its success is not dependent on describing accurately the whole.

THE RUMEN MICROBIAL ECOSYSTEM

Edited by

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15 Biological models of the rumen function

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In the preceding chapters the functions of the rumen microorganisms as a whole, in the animal or transferred to a laboratory vessel, and of microorganisms isolated from the rumen mixture acting alone or with one or two others in laboratory apparatus, have been described. In these experiments, it is assumed that the microorganisms are reproducing, *in toto* or in some specific metabolic pathway, the reactions that occur in the rumen. The results of such experiments suggested that this was indeed so and that the rumen reactions could be carried out by mixtures of a comparatively small number of species of anaerobic bacteria, the other bacteria being 'contaminants' introduced with the feed and air and being, at least in some cases, kept in small populations by continued inoculating from the surroundings of the animals. As mentioned later, the ciliate protozoa were not considered to be essential for the rumen function and the anaerobic fungi had not been recognized at the start of the experiments to be described here.

However, although experiments *in vitro* might reproduce metabolic pathways, they could not always reproduce them quantitatively and they could not demonstrate that these reactions were sufficient to keep a ruminant alive and growing; also, they could not completely reproduce the conditions in the rumen. Mechanical means cannot reproduce the rumen mixing and a dialysis membrane cannot reproduce the metabolizing rumen wall in the absorption of molecules, rumination as an aid to comminution of fibres, etc. The only 'container' that reproduces the rumen is the rumen. Thus, the idea of using the ruminant itself as a 'container' for experiments on the functions

of defined rumen floras came about. Such a ruminant can be described as a 'biological model' by analogy with the 'mathematical model' where the rumen flora is described in simplified verbal and mathematical terms.

However, in the normal animals, although the rumen is sterile at birth it is rapidly inoculated and ends up with the 'normal' rumen flora and fauna. The inoculum organisms come from the air, feedstuffs and bodily contact with nearby animals (Chapters 2, 3 and 5). While inoculation by ciliate protozoa can be prevented by keeping young animals some distance from older animals (Chapter 5), as the relatively large protozoa can travel only a short distance in air currents, rumen bacteria can be carried for long distances and can be found in samples of air from animal houses (Mann, 1963). Spatial isolation, therefore, cannot prevent the development of a rumen bacterial population in young animals. Therefore, the only way in which the rumen can be used as a model system is to isolate it, and thus the animal, in a sterile atmosphere from birth. This will keep the rumen uninoculated and so in a position to be inoculated with a population of known bacteria: to become a 'gnotobiotic' ruminant. Experiments described elsewhere (Chapters 5 and 13) have shown that the ciliate protozoa are not necessary in a rumen population that will ensure adequate growth of a young animal. On the other hand, bacteria are essential for the survival of the protozoa (Chapter 3). Since it is difficult or impossible to obtain cultures of protozoa with no, or only defined bacterial populations, experiments with gnotobiotic ruminants have concentrated on providing a defined rumen bacterial population without protozoa. Prevention of protozoal growth is also a way of manipulating the rumen microbial population, and the subject has thus been mentioned in Chapter 13.

15.1 Defined bacterial populations without protozoa

Isolation in a sterile environment is, of course, what has been done in many experiments with 'gnotobiotic' or 'germ-free' animals. There is, however, a big difference between the mice, rats, monkeys and other animals that are used in the usual gnotobiotic experiments, and ruminants. The former animals digest food by means of enzymes in gut secretions, and microbial digestion is a minor part of the digestion and not strictly necessary for the life of the animal. In the adult ruminant the main digestive process is microbial and the ruminant cannot live without a functioning rumen even if fed on a diet which could support a non-ruminant germ-free animal. Normally, the ruminant at birth has a sterile gut like any other animal, but bacteria begin to colonize the internal and external surfaces of the animal within a few minutes of birth and a gut flora soon develops (Fonty *et al.*, 1987*a*; Anderson *et al.*, 1987). This flora is initially similar to that of any other milk-fed animal, and it is not strictly necessary for digestion; so, as

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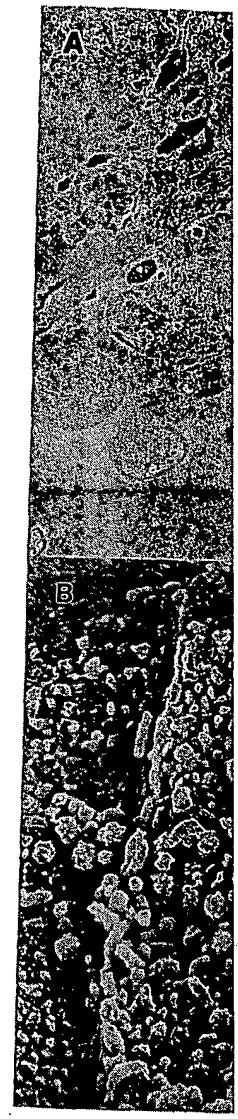
Hobson, P.N. and Stewart, C.S., The Rumen Microbial Ecosystem,
page 496 (1997, 2nd Edition, Chapman and Hall)

microorganisms are found in suspension, in association with the solid digesta, and adherent to the rumen wall (Cheng *et al.*, 1995; Figures 12.2 and 12.3), there is clearly more than one microbial compartment within this organ.

The rumen has a complex structure and the contents are heterogeneous, consisting primarily of a microbial suspension in free liquid, a solid mass of digesta, and a gas phase. Each of these entities is complex; the properties of microorganisms in suspension deep in the rumen might be different from those of microorganisms close to the rumen wall (Cheng and Costerton, 1980). The solid mass of digesta is certainly heterogeneous, and even the gas phase is complex, consisting of a large gas space (gas-cap), smaller pockets of gas entrapped within the solid mass, and the dissolved gas. Many properties of individual parts of the microbial system of the rumen have been investigated, but often the investigations were governed by the ease of experimentation rather than the importance of the given component. This is why the complex, and relatively inaccessible, semi-solid mass of digesta has received so little attention. In addition, worthwhile investigations were hampered by a lack of a simple conceptual basis for dealing with such a complex system.

The microbial populations of the rumen are effectively subdivided in Cheng's 'three population' theory (Cheng and Costerton, 1980) into those organisms free in the rumen fluid, those associated with feed particles and those associated with the rumen wall (Figures 12.2 and 12.3). Czerkawski (1980) subdivided the second population into those organisms firmly associated with particulate food (degradative compartment) and those more reversibly associated (shuttle compartment). In the functioning rumen, the compartments are clearly in a dynamic equilibrium, as ruminal microbes adhering to and detaching from their substrata (feed particles, or rumen epithelial cells) are effectively leaving and re-entering the fluid compartment.

Compartmentation within the rumen is not present immediately at birth, but evolves in the newborn ruminant over a period of approximately 20 days (Figure 12.4). Compartment 4 is the first to establish. Bacterial colonization of gut tissue in neonatal calves is initiated within 24 h of birth. Compartments 2 and 3 become established soon thereafter, often within 4 days of birth (Fonty *et al.*, 1987). Diversity of microbial members of compartments 1, 2 and 3 increases tremendously as fungal and protozoal populations become established in the rumen, at 8–10 days and 12–20 days of age, respectively. Once established, the microbial populations of each compartment are in general inherently stable and can be altered only by profound changes in nutrient substrata (e.g. transition in the ruminant's diet from forage to concentrate) or by antimicrobial agents (e.g. ionophores) which target specific populations.



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stir the contents of a round-bottomed flask. On the contrary, the raft of digesta in animals on roughage diets is more or less immobilized and the overall structure appears to favour minimal movement of the solid mass of digesta. This must be advantageous, because it is unlikely to disturb the balance of the animal, in which the rumen contents may account for up to one-fifth of its weight and whose survival in the wild state depends so much on its agility. The immobilization of the raft of fibrous digesta and its use as a continuously renewable double-sided filter (Czerkawski, 1986*b*) ensures its controlled and predictable throughput in an incredibly complicated organ. The compartmentation of the microbial population in the rumen is an indispensable and integral part of this system.

In multicellular organisms, the cells differentiate in response to the function of a particular tissue. Some cells remain fixed to form definite functional structures (e.g. muscle cells), while others become mobile (e.g. blood cells). The integrity of this complex system is maintained by connective tissues. The microbial system of the rumen is not a simple fermentation vat filled with randomly distributed mixtures of microorganisms. It is a highly structured and functionally compartmented system, on a par with any of the organs of the advanced multicellular organism. Significant progress in the field of rumen metabolism is contingent on appreciation of the rumen and its microbial ecosystem in this manner.

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page 428 (1988, 1st Edition, Elsevier Science Publishers Ltd.)

optimization. Compounds resulting in a generally lower microbial fermentation activity in the rumen are not interesting, and a high degree of specificity is desirable. However, the realization that the rumen fermentation is a very complex system of interrelated reactions makes a specific action by a particular additive almost impossible to obtain. A compound may possibly have a very specific direct effect, but the overall effect will be dictated by the changed interrelations within the whole system of rumen and indeed ruminant metabolism. For example, the use of methane inhibitors such as chloral hydrate or BES, specifically toxic only for the unique group of methane bacteria, induces a chain reaction with final effects on the pattern of volatile fatty acid production and even on degradation of certain amino acids.

It is striking that, with practically all additives (rumen manipulators), reducing equivalents (2H) are diverted from methane to propionate, and this is associated with effects on amino acid metabolism. This also explains why, in most manipulated situations, acetic acid increases are rarely seen. The complexity of interrelations within the system of rumen metabolism also includes microbial growth yield and digesta kinetics, which makes the study of rumen optimization a very laborious task. Furthermore, not only the effects on the system of rumen metabolism have to be considered but, because the sites of digestion are often changed, the effects on lower-digestive-tract metabolism should be determined, as well as possible effects at the ruminant tissue level. As adaptation to certain compounds has been observed, so effects *in vivo*, including feedlot performance, should be studied in long-term experiments. When experiments also include toxicological and even ecological aspects, the burden of the experiments for both researcher and experimental animal (multiple cannulae in the digestive tract) becomes considerable. Table 1 shows that there is ample choice of compounds interfering with rumen metabolism; for most compounds, however, experimental data on actions are limited and sometimes contradictory. The complexity of the rumen and the ruminant systems to be manipulated is the main reason for variability and contradiction in experimental results. Much more work to comprehend and rationalize this complexity is needed before safe and efficient application of rumen manipulation can be considered and recommended.

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THE RUMEN MICROBIAL ECOSYSTEM

Edited by

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EXHIBIT H

of

DECLARATION UNDER 35 U.S.C. § 132

Hobson, P.N. and Stewart C.S., The Rumen Microbial Ecosystem, pages 586-587 (1997, 2nd Edition, Chapman and Hall)

digestion, microbial protein synthesis and vitamin B_{12} synthesis, has been documented by single additions of minerals to fermentations *in vitro* (Durand and Kawashima, 1980). It is difficult to extrapolate the *in vitro* observations to *in vivo* situations, because of the influence of a number of other factors, such as solubility of minerals, adaptability of microbes, and interaction among minerals.

Manipulation of ruminal fermentation with the use of minerals requires caution, because the margin between optimal, stimulatory concentration and inhibitory concentration is narrow (Mackie and Therion, 1984; Durand and Komisarczuk, 1988). Additionally, excessive concentration of one mineral may affect the utilization of other minerals by the microbes and the host.

Four- and five-carbon VFAs, phenylpropanoic and phenylacetic acids. Isobutyric acid (IBA), isovaleric acid (IVA), 2-methylbutyric acid (2MBA) (branched-chain) and valerate (straight-chain), popularly called 'isoacids', are required absolutely by or stimulate growth of several rumen bacterial species, particularly the fibrolytic organisms (Allison, 1963; Bryant, 1973). These acids, IBA, IVA, 2-MBA and valerate, are fermentation products of valine, leucine, isoleucine and proline, respectively (Allison, 1963). Valerate could also be produced from carbohydrate fermentation. Cellulolytics and some non-cellulolytics are incapable of taking up the branched-chain amino acids and, therefore, must synthesize them from ammonia and branched-chain VFAs (Dehority *et al.*, 1967). Also, these acids serve as precursors for the biosynthesis of branched, long-chain fatty acids and aldehydes of the membrane lipids of bacteria (Bryant, 1973).

Several *in vitro* fermentation studies have shown the importance of the isoacids in fiber digestion (Andries *et al.*, 1987). Addition of branched-chain amino acids (leucine and isoleucine) has also been shown to stimulate fiber

Table 13.20 Effects of carbon-4 and carbon-5 fatty acids (straight or branched) on *in vitro* wheat straw cell wall digestion and ammonia concentration

Addition	Cell wall digestion (%)	Ammonia concentration ($mg\,l^{-1}$)
None	17.1^a	177.8^b
Valeric	15.5^a	185.0^b
Isovaleric	25.4^b	162.1^a
Isobutyric	25.4^b	153.4^a
2-Methylbutyric	26.6^b	149.5^a
All four acids	26.4^b	153.2^a
SE ($n = 3$)	0.30	2.73

Data from Gorosito *et al.* (1985).
Each acid was added at 1.76 mM.
^{a,b} Means within a column with different superscripts differ ($P < 0.05$).

Table 13.21 Effects of carbon-4 (C_4) and carbon-5 (C_5) fatty acids (straight or branched) on the plant cell wall digestion from intact substrates[a]

Substrates	Control[b]	C_4 and C_5 acids[c]	Significance[d]
Alfalfa hay	34.1	38.7	*
Timothy hay	48.5	52.6	NS
Wheat straw	10.4	13.0	**
Reed canary grass	32.1	37.8	NS
Bermuda grass	18.2	21.8	NS
Orchard grass	30.2	38.0	*
Corn silage	34.1	36.7	*
Filter paper[e]	1.3	11.9	**

[a] Expressed as percentage dry matter.
[b] No C_4 and C_5 acids were added.
[c] Each acid (valeric, isovaleric, isobutyric and 2-methylbutyric) was added at 1.76 mM.
[d] Means within a row differ (*$P < 0.05$ or **$P < 0.01$); NS, not significant ($P > 0.05$).
[e] Ball milled Whatman no. 1 filter paper.
Data from Gorosito *et al.* (1985).

digestion, although valine alone had no effect in stimulating barley straw digestibility (Mir *et al.*, 1986). Incubations of mixed ruminal bacteria and isolated plant cell walls or intact forages with isoacids resulted in increased cell wall digestibility and ammonia assimilation (Table 13.20; Gorosito *et al.*, 1985). Such positive responses *in vitro* were obtained only with the branched-chain fatty acids and not with valerate (Cummins and Papas, 1985; Gorosito *et al.*, 1985). Also, the stimulatory response was not observed with all forage sources and apparently was not related to the protein content (Table 13.21; Gorosito *et al.*, 1985). For example, the highest stimulation was observed with filter paper, a pure cellulose substrate; however, a significant response was also obtained with alfalfa, a forage with high protein content (Gorosito *et al.*, 1985; Mir *et al.*, 1986). The stimulation of bacterial activity was also evidenced by increased VFA production (Kone *et al.*, 1989) and bacterial protein synthesis (Russell and Sniffen, 1984; Andries *et al.*, 1987).

In contrast to the studies *in vitro*, the results of supplementation *in vivo* on fiber digestibility and microbial protein production have been minimal or non-significant (Andries *et al.*, 1987). Gunter *et al.* (1990) observed no significant effect on fermentation and microbial protein synthesis in the rumen of beef heifers consuming grass hay supplemented with branched-chain fatty acids. Several investigators have studied the influence of supplementation with isoacids on the performance of growing cattle and dairy cows (Andries *et al.*, 1987). In high-yielding cows, supplementation with isoacids seems to increase milk production. Papas *et al.* (1984) reported that, in four of five trials, such supplementation increased the 305-day milk production by 4–8% in cows. Milk composition was not affected. In contrast, the response with growing cattle was minimal and suggested that

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EXHIBIT I

of

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Hobson, P.N., The Rumen Microbial Ecosystem,
pages 140-141 (1988, 1st Edition, Elsevier Science Publishers Ltd.)

Methanogenic bacteria sometimes contaminate fresh isolates of rumen anaerobic fungi (Bauchop & Mountfort, 1981), but they can be removed by treatment with chloramphenicol.

When rumen fungi are cultured axenically on plant fibre, rhizoid tissue may form a mat on the surface of the fibre (Fig. 4). This is in contrast to fungi growing in the rumen where fungal rhizoids are located beneath the surface of the plant tissue.

Culture media

The most commonly employed, undefined culture media used for growing anaerobic rumen fungi are similar to that of Orpin (1975), and consist of centrifuged rumen fluid, tryptone, yeast extract, a carbon source, a carbon dioxide–bicarbonate buffer at pH 6·7–6·9, L-cysteine as reducing agent, and vitamins. This medium may be solidified with 0·8–1·5% agar for use in isolating clones from single colonies (Joblin, 1981). If bacterial contamination of cultures occurs, antibiotics such as ampicillin, streptomycin and chloramphenicol may be incorporated into the medium.

Some strains of the polyflagellated species have been grown in defined media, for example *N. patriciarum* (Orpin & Greenwood, 1986*b*) and unidentified *Neocallimastix* spp. (Lowe *et al.*, 1985).

Culture media incorporating plant tissues as carbon source are valuable for ensuring that the organisms do not lose their ability to ferment plant structural carbohydrates *in vitro* (Orpin & Letcher, 1979). Cultures in the liquid media of Orpin (1975) gave high cell yields but needed subculturing after 24–48 hours to maintain viability, whereas cultures on plant tissues may be viable for up to 7 days, thus simplifying routine maintenance. Maintenance of cultures for periods of several months without subculturing has been achieved with *Neocallimastix* and *Piromonas* spp. by the storage at 39°C of cultures on plant tissues embedded in agar (Joblin, 1981). Long-term storage has been achieved by controlled freezing in liquid nitrogen or at −70°C using 5% dimethyl sulphoxide as cryoprotectant (Yarlett *et al.*, 1986*b*).

Nutrition

Only one species, *Neocallimastix patriciarum*, has been grown in a minimal medium (Orpin & Greenwood, 1986*b*). Minimal nutritional requirements for growth of *N. patriciarum* on cellobiose in a CO_2 atmosphere were satisfied by the provision of sources of haem, D-biotin, thiamin or its precursors, ammonium ions, and of reduced sulphur and trace elements. Growth was stimulated by amino acids, straight and branched short-chain volatile fatty acids, low concentrations of long-chain fatty acids and a number of vitamins. Germination of zoospores was stimulated by acetic acid and soluble, fermentable carbohydrates.

Haems appear to play a major role in both the nutrition and zoosporogenesis of rumen fungi (Orpin, 1986*a*). Although haems probably occur in rumen contents during the entire day, the addition of haems to rumen contents of animals fed once per day resulted in zoosporogenesis of *Neocallimastix* sp., but this could not be satisfactorily repeated *in vitro* with pure cultures, suggesting that the control of

zoosporogenesis and zoospore release may be more complex than suggested by the work *in vivo*. Haems may act synergistically with other dietary components to stimulate zoosporogenesis. Because of the low redox potential of the rumen, dietary haems in an oxidised state would be rapidly reduced, and it is likely that the reduced rather than the oxidised haem is functional *in vivo*. Zoosporogenesis in monoflagellated species may also be triggered by similar compounds (Orpin, 1977*d*).

INTERMEDIARY METABOLISM

In *Neocallimastix patriciarum* the Embden–Meyerhof pathway appears to be the major glycolytic pathway used in the catabolism of carbohydrates (Yarlett *et al.*, 1986*a*). This is supported by the distribution of $^{14}CO_2$ produced by cultures fermenting glucose which had been labelled in C-1, C-2 and C-6 positions (Orpin, C. G., unpublished).

Glycolysis generates phosphoenolpyruvate which is converted via oxaloacetate into malate. Hydrogenosomal enzymes generate energy from the oxidation of malate to pyruvate which is coupled, via NADPH:ferridoxin oxidoreductase, ferridoxin and hydrogenase, to production of molecular hydrogen. Some of the pyruvate generated is converted into acetyl-CoA via pyruvate:ferridoxin oxidoreductase, and subsequently into acetate. Cytosolic lactate dehydrogenase is responsible for lactate generation and, when cultures are grown under a N_2 rather than under a CO_2 atmosphere, low levels of alcohol dehydrogenase convert acetaldehyde into ethanol (Yarlet *et al.*, 1986*a*). Carbon dioxide appears to suppress hydrogenosomal acetate generation as well as cytosolic ethanol production, perhaps by end-product inhibition of the pyruvate:ferridoxin oxidoreductase and pyruvate decarboxylase reactions, both of which generate CO_2. One strain of *Neocallimastix* was found to require 7% CO_2 in the gas phase and could not be grown under a 100% N_2 atmosphere (Joblin, K., unpublished).

FERMENTATION OF CARBOHYDRATES

It is clear that the rumen fungi so far examined produce a wide range of enzymes that can digest the major structural carbohydrates of plant cell walls (Pearce & Bauchop, 1985; Wood *et al.*, 1986; Williams & Orpin, 1987*a*; Lowe *et al.*, 1987*b*,*c*), hydrolyse a range of glycosidic linkages (Williams & Orpin, 1987*b*), and enable the fungi to grow on a number of polysaccharides (Orpin & Letcher, 1979; Mountfort & Asher, 1983). Many of the polysaccharide-hydrolysing enzymes are produced by the vegetative stage and the zoospores of the fungi, and are also present in the extracellular culture medium (Williams & Orpin, 1987*a*,*b*; Lowe *et al.*, 1987*b*). The particular activity depends upon the growth substrate. Thus *Neocallimastix frontalis* and *N. patriciarum* will utilise cellulose, xylan, starch and hemicelluloses (Orpin & Letcher, 1979; Bauchop, 1983; Orpin & Munn, 1986), as will *P. communis* and another monoflagellated species (Williams O Orpin, 1987*a*). No

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Santoro, Luiz G., Grant, George, and Pusztai, A. Arpad, Differences in the Degradation *In Vivo* and *In Vitro* of Phaseolin, the Major Storage Protein of *Phaseolus Vulgaris* Seeds, pages 612-613, Biological Society Transactions - 625th meeting, (London 1998)

phonic analogue is not recognized. These collected findings are consistent with recent crystallographic and chemical studies on the active site of the enzyme (Sandmeier & Christen, 1982; Arnone *et al.*, 1984; Kirsch *et al.*, 1984): the substrate specificity is largely determined by the interactions of α- and ω-carboxylate groups with guanidinium groups of Arg-386 and Arg-292, respectively. The binding of the two carboxylate groups induces a conformational change of the protein, and the ε-amino group of Lys-258 which binds the coenzyme can act as C_α–H proton acceptor. It may be assumed that the replacement of one of the carboxylate groups induces restrictions in such conformational changes, leading to a decrease in reactivity of the effector. Distinctions in cytosolic and mitochondrial isozymes with respect to the distances between the two arginyl residues and the lysyl 258 Schiff's base, and/or the degree of freedom of the rotation of these active site components (Iriarte *et al.*, 1984) could explain the different data observed for Asp-β-P and Glu-γ-P with the two isozymes.

The present results relative to the aminomalonate analogue and Asp-β-P, which are both substrates and inhibitors, show the importance of an α-carboxylic group in the binding of an effector with the ASAT active site.

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Differences in the degradation *in vivo* and *in vitro* of phaseolin, the major storage protein of *Phaseolus vulgaris* seeds

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Storage proteins of legume seeds are considered to be poorly digestible in their native form (Liener & Thompson, 1980). The partial resistance of native phaseolin (glycoprotein II, G-II), the main storage protein of *Phaseolus vulgaris*, to degradation by pepsin, trypsin and chymotrypsin, *in vitro*, alone or in a sequential combination (Liener & Thompson, 1980; Deshpande & Nielsen, 1987) has been demonstrated. Limited proteolysis by trypsin or chymotrypsin reduced the size of the native protein from 140 kDa to 120 kDa (Liener & Thompson, 1980). The subunits of G-II were cleaved near the middle of the polypeptide chains and the products in SDS had M_r values in the range of 22–30 kDa. According to Vaintraub *et al.* (1976) and R. Begbie (unpublished work), the hydrolysis *in vitro* of undenatured G-II by pepsin resulted in only about 2% of the total peptide bonds being broken. On the other hand, G-II appeared to be more extensively degraded *in vitro*, if instead of pure gut endopeptidases, stomach and small intestinal luminal and tissue extracts were used as sources of proteolytic enzymes (Sgarbieri *et al.*, 1982).

As there is much less information available about the degradation *in vivo* of pure G-II, in the present work, the true digestibility *in vivo* of a highly purified G-II preparation was studied in rats.

G-II was isolated from seeds of *P. vulgaris* as before (Pusztai & Watt, 1970). The amount of lectin contamination was reduced to less than 0.3% by affinity chromatography on Sepharose 4B-fetuin. It was thought unlikely that the results would be influenced unduly by the presence of such a small amount of toxic component.

Rats fasted for 16 h were given an intragastric dose of 300 mg of G-II (45 mg N) and then fed a protein-free diet, *ad lib*, for 3 days. Faeces were collected daily and N estimated. Faeces were also extracted with 0.025 M-glycine/Tris buffer, pH 8.6 (3–5 mg of faeces/ml of buffer) and the concentration of G-II-related material was estimated by rocket immunoelectrophoresis. This was based upon previous observation (R. Begbie, unpublished work) that the fragment derived from the partial digestion of G-II retained full reactivity with anti-G-II antibodies.

It was found that (Fig. 1*a*) the faeces contained more than 50 mg of total N although only 45 mg of G-II N had been introduced into the stomach. The faecal N comprised approx. 12 mg of G-II N. The remainder, about 38 mg, was unrelated to G-II, indicating that 74% of the G-II was degraded during passage through gastrointestinal tract. The bulk (8 mg) of the partially digested G-II N appeared in the faeces in the first 24 h, while the output of N unrelated to G-II peaked on the second day. It appears that by an unknown mechanism, the native G-II and/or its fragments may have stimulated an increased secretion of endogenous metabolic N.

Since proteolysis by bacteria in the large intestine may have made appreciable contributions to degradation of G-II without nutritionally benefiting the rat, in another series of experiments rats were given an intragastric dose of 150 mg of G-II (22.5 mg N) and killed after 1 h. Both stomach and small intestine were removed and their contents washed out. The tissues were then homogenized in phosphate buffer containing aprotinin (5 mg tissue/ml of buffer) and centrifuged. After correction for the control (rats given an intragastric

Abbreviation used: G-II, glycoprotein II.

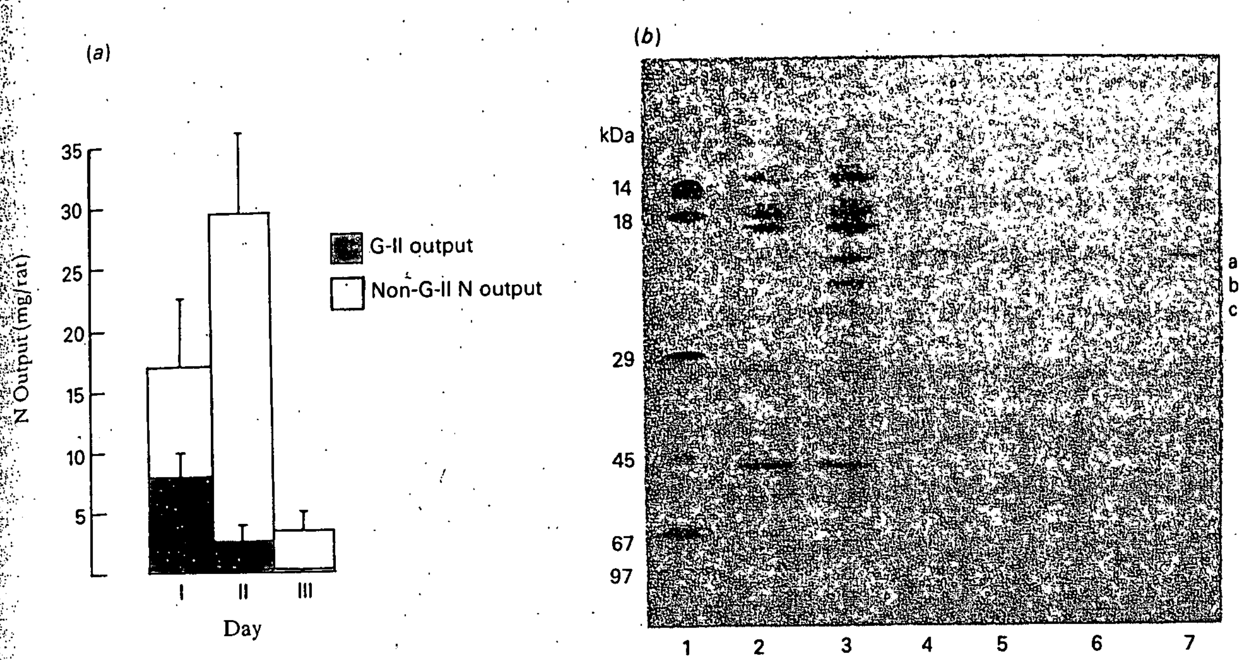

Fig. 1. *(a) Total N (corrected for normal metabolic faecal N) and G-II N output over 3 days in the faeces of rats given 300 mg of G-II (45 mg N) and (b) SDS/PAGE of G-II related polypeptides*

Lane 1, standards; lane 2, extract from the small intestine tissue of control rats; lane 3, extract from the small intestine tissue of rats given an intragastric dose of G-II; lane 4, G-II core-polypeptides recovered in luminal contents of rats given G-II; lane 5, native G-II; lanes 6 and 7, G-II core-polypeptides from faeces; (a) ≈ 22 kDa, (b) ≈ 23 kDa, (c) ≈ 25 kDa.

dose of saline), the total amount of N found in the luminal contents and tissue homogenates was at least as much (23.4 ± 0.8 mg) as in the G-II input. On the other hand, the amount of G-II-related N recovered from the small intestine was only 9.6 ± 0.8 mg, indicating that about 57% of the G-II was degraded and absorbed within 1 h. Moreover, a large proportion (about 60%) of the surviving G-II-related protein was found to be strongly associated with small intestinal tissue (Fig. 1*b*) and was released only on homogenization.

In conclusion, at least 57% of G-II was degraded in the small intestine within 1 h. During the remaining time (≈ 1 h) in the small intestine and then in the large intestine, G-II was further degraded. The core-polypeptide fragments of 22–30 kDa surviving in the small intestine showed a similar subunit pattern by SDS/polyacrylamide gel electrophoresis (PAGE) to that observed on degradation *in vitro* (Deshpande & Nielsen, 1987). As the extent of degradation of G-II *in vivo* (74%) exceeds that obtained *in vitro* by pure endopeptidases (2%), the results strongly suggest the involvement of additional proteinases in the breakdown of G-II in the gut. The strong attachment of G-II to the intestinal tissue and extended exposure time to proteolytic enzymes may further aid this digestive process. However, an appreciable part (about 26%) of the dietary G-II escapes digestion in the whole alimentary tract. The reasons for this may be related to the previously observed microheterogeneity of G-II (Pusztai & Stewart, 1980) or partial protection from proteolysis by an unknown mechanism.

Finally the large difference between apparent and true digestibility values indicates that G-II and/or its fragments are stimulants of secretion of endogenous N (mucus, etc.) in the small intestine.

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EXHIBIT K

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[54] **FODDER ADDITIVE FOR RUMINANTS**

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[57] **ABSTRACT**

A fodder additive is provided for increasing the blood sugar of ruminants and their milk level. The fodder additive comprises as active agents at least one sugar alcohol having five or six hydroxyl groups, especially xylitol, arabitol and dulcitol. A preferred additive contains, based upon dry weight: 5–25% xylitol; 20–35% arabitol; 10–25% mannitol; 5–15% sorbitol; 5–10% dulcitol; 5–10% rhamnitol.

9 Claims, No Drawings

FODDER ADDITIVE FOR RUMINANTS

BACKGROUND AND OBJECT OF THE INVENTION

The object of the invention is a fodder additive for use in the feeding of ruminants, the additive influencing advantageously the milk production of cows by increasing their blood sugar level.

The metabolism of the organism requires easily soluble carbohydrates, above all various sugars, of which glucose is the most important. In the non ruminants the large molecular carbohydrates, such as starch, split into sugars by means of the digestive fluid in the intestine. In ruminants, the same splitting takes place already in the proventriculi, above all in the rumen, through the action of microbes. These carbohydrates as well as the sugars contained in the fodder are used as an energy source of the microbes and are thus converted into a microbial mass and consequently do not directly increase the sugar supply to the organism.

Animals, including ruminants, need sugar predominantly for the function of the liver and the mammary glands. Glucose is converted into lactose in the cow's udder and is secreted together with the milk. In a cow yielding 30 kg of mild daily this corresponds to about 1500 g of glucose leaving the organism daily. Taking into consideration the animal's further requirements it is estimated that a medium size cow in its period of maximum production requires about 2000 g/day of sugar. This sugar amount must be supplied by the liver. As no considerable amounts of sugar, especially glucose, pass through the rumen, intact glucose must be produced in the liver of the ruminants. Important glucose sources constitute propionic acid in the rumen and glucose synthesis by the organism from proteins and lactic acid. It is however estimated that the sugar deficit in an animal yielding 30 kg of milk daily is about 700 g. The animal attempts to compensate for this deficit by breaking down organism fats. By this means glycerol is formed which is converted into glucose. Also fatty acids are formed, which in turn break down into ketone-bodies. Thus, the animal might suffer from ketosis in case an excessive degradation of fats occurs.

If the supply of glucose to the animal is ensured, the breaking down of fat tissue decreases which, however, is not normally sufficient to maintain the glucose level in the blood.

The glucose level in the udder plays an essential role in the regulation of the mild yield. If the udder tissue contains large amounts of glucose, the cells will absorb fluid by osmosis from the blood to compensate the osmotic pressure. The milk yield thus increases. If the sugar content is low, the opposite phenomenon occurs.

THE PRIOR ART

As today's high yield ruminants are not always capable of ensuring their glucose need, auxiliary feeding means have been used. Ruminants have been administered glucogenic feeding additives which do not break down in the rumen, but pass intact therethrough to the later stomach compartments and from there on to the liver, where they are converted into sugars. It is also possible to try to increase the propionic acid content in the rumen as propionic acid is converted into active glucose in the liver. For this purpose it is known to use the following substances: propylene glycol, glycerol, various propionates, even propionic acid.

SUMMARY OF THE INVENTION

According to the invention it has now surprisingly been found that an additive to be incorporated into ruminant fodder and exhibiting a high sugar alcohol content, an advantageous composition of which is found in the mixture of various sugar alcohols obtained as a by-product in the production of xylitol from plant material, substantially improves the glucose balance of ruminants as it acts similarly to the above mentioned glucogenic substances. The characteristics of the additive appear in the appended claims.

An advantageous composition contains xylitol, arabitol, dulcitol and possibly rhamnitol.

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The product according to the invention is called a sugar alcohol mixture, one embodiment of which is obtained as a by-product of the xylitol production from birch. It is a yellowish brown sweet fluid exhibiting a specific weight of about 1.22 as a 50 percent aqueous solution at room temperature. Its energy (caloric) value and degree of sweetness are same as for glucose. A composition of the product in question was earlier approximately the following, calculated on a dry matter basis:

xylitol	15–25 %	by weight
arabitol	20–35 %	"
mannitol	15–25 %	"
sorbitol	5–15 %	"
dulcitol	5–15 %	"
rhamnitol	5–10 %	"
others	2–5 %	"
break-down products	2–5 %	"

In more recent processes the recovery of xylitol has been improved and the composition may vary within the following limits:

xylitol	6–18 %	by weight
arabitol	9–21 %	"
mannitol	13–19 %	"
sorbitol	8–12 %	"
dulcitol	5–11 %	"
rhamnitol	4–6 %	"
reducing sugars	8–15 %	"
other polyols	6–14 %	

It is known from literature that xylitol stimulates the liver functions and enhances the cell activity. It is also known that the microbes of the mouth are not able to utilize xylitol as an energy source. The present invention, however, is based upon the prior unknown fact that sugar alcohols remain intact also under the conditions in the rumen sufficiently long without breaking down, which appears from the test results presented in Example 1.

EXAMPLE 1

In Example 1 the breaking down of various single sugar alcohols in the rumen has been studied in vitro.

The preservation of sugar alcohols in the rumen fluid (% of the amount added)

Incubation time	2h	4h	8h	24h	48h
xylitol	91.2	89.7	88.4	83.4	33.8
arabitol	86.8	93.0	88.3	87.0	55.9
mannitol	88.9	88.1	80.5	1.4	0.4

-continued

Incubation time	2h	4h	8h	24h	48h
dulcitol	92.9	94.3	85.8	61.2	7.0
sorbitol	91.3	88.3	79.6	11.1	0.2

It is concluded that the sugar alcohols in question remain almost completely intact without being metabolized for 8 hours, xylitol, arabitol and dulcitol even for 24 hours and arabitol and xylitol partly even longer. Ordinary sugar breaks down in less than 2 hours.

EXAMPLE 2

This Example concerns the behaviour in the rumen fluid of a sugar alcohol mixture of the following composition calculated as dry matter

xylitol	18 %	by weight
arabitol	24 %	"
mannitol	18 %	"
sorbitol	9 %	"
galaxitol	7 %	"
rhamnitol	7 %	"
reducing sugars	7 %	"
other polyols	10 %	"

and it is concluded that its preservation is comparable to the preservation of the single sugar alcohols. Incubation time 2h 4h 8h 24h 48h % left of the amount added 92.6 86.7 87.6 55.2 23.7

As the flow of the fluid in the rumen is only a few hours, the sugar alcohol reaches the latter stomach compartments before any essential degradation in the rumen can occur.

After passing through the rumen the sugar alcohols act according to the normal sugar metabolism, which is evidenced i.a. as an increase in the blood glucose level as shown in Example 3.

EXAMPLE 3

Changes in the blood glucose level

days after start of test	0	10	13	15	17	18	19	21	25
	Test period								
					Sugar alcohol				
No sugar alcohol					2 × 125 g dry matter		2 × 250 g dry matter		
glucose mmol/l	3.4	3.1	3.0	2.5	2.7	3.1	3.2	3.2	3.5

During normal feeding the glucose level of the test animal decreased constantly, and was less than 3.0 mmol/l already before starting the test feeding. The administration of 2 × 125 g dry matter of the sugar alcohol mixture according to Example 2 was begun on the 17th day of the test period. The blood glucose values showed an increase the very same day. After one week of test feeding the glucose value re-established its initial value at the beginning of the test period. The animal's general condition returned to normal, as judged externally.

The fat percentage in the milk of the test animal varied before administration of the sugar alcohol mixture by almost 2 percentage units daily, being at an average 4.5%. After starting the test feeding the fat percentage decreased within two days to the level specific for the animal in question, i.e. below 4.0%. Simultaneously the daily fluctuations became insignificant.

The energy and above all the sugar metabolism of the animal of Example 3 was disturbed to such a degree that ketose would have been imminent had not the test feeding been started and the sugar metabolism restored to normal.

By acting on the rumen functions by increasing the propionic acid production at the expense of acetic acid and butyric acid, the sugar metabolism of ruminants may be improved, as propionic acid is converted to extremely effective glucose in the organism.

Example 4 illustrates the effect of a sugar alcohol mixture on the fatty acid production of the rumen. An increase in the acetic acid content means an energy waste, as its further metabolization gives rise to carbon dioxide which leaves the organism unused. On the other hand, propionic acid is converted entirely into glucose.

EXAMPLE 4

Rumen specimens obtained with a fistula were investigated as to the changes in the acetic acid (E) and propionic acid (Pr) contents of the rumen fluid. During the pre-feeding stage the percentage of E showed an increase whereas that of Pr decreased. After starting the test feeding the ratio began to change into the opposite direction. Externally judged, the animal visibly improved.

Test period	1	2	3	4	5	6
E %	51	60	62	57	51	52
Pr %	28	26	25	27	28	29

Test stages 1 to 3 correspond to winter feeding with standard fodder, whereby symptoms of inbalance in the sugar metabolism were evident during feeding, at stage 4 the cow was fed a sugar alcohol mixture the composition of which corresponded to Example 2, in an amount of 2 × 125 g, at stage 5 the dose was 2 × 250 g and at stage 6 the dose was 2 × 200 g of sugar alcohols daily calculated as dry matter per animal.

It is concluded that the propionic acid formation by the rumen increased by more than 100 g daily as a result of the sugar alcohol mixture. The major part of the sugar alcohol mixture however passes through the rumen without breaking down.

EXAMPLE 5

A cow that had already passed its high-yeilding stage after calving was administered a solution containing sugar alcohols (c.f Example 2) at an amount of 0.4 liters daily. The composition of the solution was:

propylene glycol	10 %	by weight
sodium propionate	5 %	"
sugar alcohol mixture (50 % dry matter)	40 %	"
molasses (60 % dry matter)	45 %	"
	100 %	(~ 65 % dry matter)

The milk yield during the test feeding which lasted for about 4 weeks increased clearly, the increase being 0.1 kg daily. The test animal had already passed its peak yeild at the beginning of the test feeding, the energy requirement as glucose thus having already decreased from its maximum value. Depsite this the animal incrased its milk production. A similar test cannot be

carried ut during the early lactation, as it is not possible to say h w much of the production increase is due to the normal increasing of the yield and how much is due to the fodder additive.

As a vehicle or fodder component for the additive mixture e.g. a by or waste product of a nutrient production process, such as molasses, is advantageously used. Into the mixture may also be incorporated glucogenic substances known per se, such as propylene glycol, whereby the amount of sugar alcohol mixture may be correspondingly reduced. The amount of sugar alcohol mixture is, however, always at least 10% by weight calculated on the dry matter of the mixture.

The additive mixture according to the invention may be used as a solution, whereby it may be packed in suitable containers and administered directly during feeding. The recommended dosage is 40 to 500 g of sugar alcohol daily calculated on the dry matter, per animal. The additive may also be dried and added e.g. to other industrial fodders, or used as such as dry bulk feeds.

I claim:

1. Method of increasing milk yield in a ruminant which comprises enriching fodder for said ruminant with an additive consisting essentially of a sugar alcohol having five or six hydroxyl groups to the fodder of the ruminant in an amount of at least 10% by weight calculated on the dry matter of fodder and feeding said enriched fodder to the ruminant in an am unt sufficient to administer 40 t 500 grams daily of said sugar alcoh ls to said ruminant, whereby the milk yield from said ruminant is increased.

2. Method according to claim 1, wherein said additive contains a member selected from the group consisting of xylitol, arabitol, dulcitol and mixtures thereof.

3. Method according to claim 1, wherein the sugar alcohols comprise by-products obtained from the xylitol production from plant material.

4. Method according to claim 3, wherein the additive contains, calculated on the weight of dry matter, xylitol 5 to 25% arabitol 20 to 35%, mannitol 10 to 25%, sorbitol 5 to 15% dulcitol 5 to 10%, and rhamnitol 5 to 10%.

5. Method according to claim 1, wherein the fodder comprises a by-product from a nutrient production process, such as molasses.

6. Method according to claim 1, wherein said additive further contains a glucogenic substance.

7. Method according to claim 6, wherein said glucogenic substance is propylene glycol.

8. Method according to claim 1, wherein said additive is in the form of a solution.

9. Method according to claim 1, wherein said additive is in the dried form.

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